



US005266463A

**United States Patent** [19]**Takahashi et al.**[11] **Patent Number:** **5,266,463**[45] **Date of Patent:** **Nov. 30, 1993**[54] **HIGHLY SENSITIVE ASSAY METHOD FOR L-CARNITINE AND COMPOSITION FOR PRACTICING SAME**[75] Inventors: **Mamoru Takahashi; Shigeru Ueda**, both of Shizuoka, Japan[73] Assignee: **Asahi Kasei Kogyo Kabushiki Kaisha**, Osaka, Japan[21] Appl. No.: **640,117**[22] Filed: **Jan. 11, 1991**[30] **Foreign Application Priority Data**

Jan. 11, 1990 [JP] Japan ..... 2-4063

Nov. 9, 1990 [JP] Japan ..... 2-305439

[51] Int. Cl.<sup>5</sup> ..... **C12Q 1/32; C12P 19/36**[52] U.S. Cl. .... **435/26; 435/89; 435/90; 435/117; 435/128; 435/829; 436/815**[58] Field of Search ..... **435/26, 89, 90, 117, 435/128, 829; 436/815**[56] **References Cited****U.S. PATENT DOCUMENTS**

3,964,974 6/1976 Banauch et al. .... 435/26

4,221,869 9/1980 Vandecasteele et al. .... 435/117

4,598,042 7/1986 Self ..... 435/26

4,650,759 3/1987 Yokozeki et al. .... 435/128

**OTHER PUBLICATIONS**

"A Simple Fluorometric Method for the Determination of Serum Free Carnitine", by K. Watanabe et al., pp. 315-318.

"A Method for the Fractionation and Determination of Carnitines in Milk and Milk Products", by M. Hamamoto et al., pp. 389-395.

"Quantitative Bestimmung von L-Carnitin mit Hilfe von Carnitin-dehydrogenase aus *Pseudomonas putida*", by W. Schopp et al., pp. 285-289."An Improved and Simplified Radioisotopic Assay for the Determination of Free and Esterified Carnitine", *Journal of Lipid Research*, vol. 17, 1976, by J. McGarry et al., pp. 277-281."A Method for the Determination of Carnitine in the the Picomole Range", *Clin. Chim. Acta*, vol. 37, 1972, by G. Cederblad et al., pp. 235-243.

"Enzymological Determination of Free Carnitine Con-

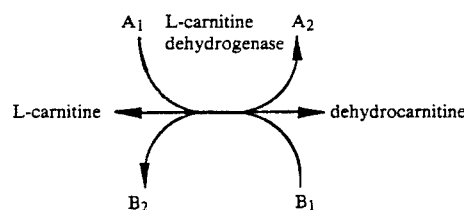
centrations in Rat Tissues", *Journal of Lipid Research*, vol. 5, 1964, by N. Marquis et al., pp. 184-187."Properties of Partially Purified Carnitine Acetyltransferase", *The Journal of Biological Chemistry*, vol. 238, No. 7, Jul. 1963, By I. Fritz et al., pp. 2509-2517.Schopp et al., *European J. Biochem.*, vol. 10, pp. 56-60, 1969.Aurich et al., *European J. Biochem.*, vol. 6, pp. 196-201, 1968.Matsumoto et al., *Clin. Chem.*, vol. 36, No. 12, pp. 2072-2076, 1990.*Primary Examiner*—Michael G. Wityshyn*Assistant Examiner*—Abdel A. Mohamed*Attorney, Agent, or Firm*—Young & Thompson[57] **ABSTRACT**

A method of assaying L-carnitine in a specimen comprises reacting a specimen containing L-carnitine with:

a) L-carnitine dehydrogenase having coenzymes of the thio-NAD group and of the NAD group, and which catalyzes a reversible reaction forming dehydrocarnitine from a substrate of carnitine,

b) A<sub>1</sub> andc) B<sub>1</sub>

to effect a cycling reaction of the formula



wherein A<sub>1</sub> is thio-NAD group or NAD group, A<sub>2</sub> is a reduced form of A<sub>1</sub>, when A<sub>1</sub> is thio-NAD group, B<sub>1</sub> is reduced NAD group and when A<sub>1</sub> is NAD group, B<sub>1</sub> is reduced thio-NAD, and wherein B<sub>2</sub> is an oxidized form of B<sub>1</sub>; and measuring an amount of A<sub>2</sub> or B<sub>1</sub> generated or consumed by the cycling reaction. A composition for performing the assay comprises the above L-carnitine dehydrogenase, as well as the above components A<sub>1</sub> and B<sub>1</sub>.

**10 Claims, 2 Drawing Sheets**